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APPENDIX 1: Protocol for Setting up the Assay Using ibidi Plates

1. Each lab group will utilize two ibidi plates, one for treatment with DMSO (1% FBS DMEM + DMSO; control) and the other for drug treatment (1% FBS DMEM + drug of choice dissolved in DMSO; experimental). Be careful when handling as these are small plates.
2. Label your plates properly on the bottom, not the lid, with your group initials and the type of treatment that will be used on each plate.
3. Prepare the culture-insert according to the manufacturer's protocol.
4. Seed appropriate number of cells and wait for cell attachment. Detailed step-by-step information about HeLa cell culture can be found in our previous publication (4).
5. When cells are attached, gently remove lid. Using a pair of tweezers sterilized with 70% ethanol, remove the plastic grid in the middle of the plate by pulling upwards carefully.
6. Tilt the plate to make the media pool, and gently remove the media using a micropipette. Do not aspirate with vacuum as it is difficult to control suction capacity.
7. Gently add fresh 2 mL 1% FBS DMEM to the side of the plate and avoid pouring the media on the area where cells are located which will dislodge the cells.
8. Check the plate under a microscope to see if the cell quadrants are well-defined and if the plate is free of non-adherent cells. Remove media using a micropipette.
9. Repeat rinsing the plate with media until there are no floating cells and the cell quadrants are well-defined.
10. Add 2 mL of media of choice (1% FBS DMEM containing either DMSO or drug) and incubate the plate in the incubator.
11. Monitor cell migration and wound closure over time, and record images of the cells as necessary.

APPENDIX 2: Wound Healing Data Analysis Guide

When students took pictures of the wound under microscope, the images were not oriented properly. Therefore, each picture was aligned using ImageJ software (*Image* > *Transform* > *Rotate*) so that the center gap is in the middle. Then the center area was selected using the "rectangle" tool, followed by cropping the area (*Image* > *Crop*). For analysis, we first converted the image to grayscale (*Images* > *Type* > *8-bit*), found edges (*Process* > *Find Edges*), and then blurred the image multiple times (*Process* > *Smooth*) so that the area containing cells were white whereas

the acellular area remained black. Next, MinError threshold was applied (*Image > Adjust > Auto Threshold: MinError*) to detect the area not covered by the cells. We then used the Analyze menu to measure the cell free area (*Analyze > Set Measurement > Area: Limit to threshold*). Finally, we selected (*Analyze > Measure*) which will measure the area in square pixels. If the software measures the cellular area, the wound area can be easily calculated by subtracting the cellular area from the total area. The total area of an image can be obtained by multiplying the number of horizontal and vertical pixels. For details about wound healing data analysis using ImageJ, please refer to (5). Finally, the percentage wound closure was calculated using the following equation (7):

$$\% \text{ Wound Closure} = \frac{(\text{Wound area at 0 hr} - \text{Wound area at X hr})}{\text{Wound area at 0 hr}} \times 100$$